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Accelerated Degradation and Mineralization of Atrazine in Surface and Subsurface Soil Materials*

Vincent Vanderheyden, Philippe Debongnie & Luc Pussemier‡

Institute for Chemical Research, Leuvensesteenweg 17, 3080 Tervuren, Belgium

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Abstract: In surface soils, atrazine is considered to be a moderately persistent herbicide, with half-lives ranging generally from one to two months. In subsoils, however, its degradation is generally slower. This paper reports the degradation of atrazine in soil and subsoil samples taken from six Belgian maize fields. Rapid degradation can take place in some samples taken from surface and in some from subsurface soils. Subsoil samples were found to degrade atrazine either very strongly or not at all. Experiments with [*ring-U*-^{14}C] atrazine showed that the micro-organisms responsible for the rapid degradation cleave the triazine ring and extensively mineralize the molecule.

Key words: atrazine, soil, subsoil, biodegradation, mineralization

1 INTRODUCTION

The herbicide atrazine has been widely used in agriculture and in urban areas and this widespread use has caused much contamination of groundwaters. In Europe, atrazine is often detected in surface- and groundwaters at concentrations exceeding the maximum pesticide contaminant level of 0·1 μg litre^{-1} set by the European Union.

Atrazine is a relatively persistent herbicide with half-lives in surface soils ranging generally from some weeks to some months. After leaching beneath the cultivated horizon, it is only the ability of subsoils to detoxify, adsorb or immobilize the pesticide that can prevent the contamination of groundwater.

For surface soils it is well known that micro-organisms play a determining role in the degradation of pesticides, but little is known about the fate of xenobiotic compounds in subsoils. This lack of data and the numerous cases of pollution by pesticides have stimulated studies dealing with pesticide degradation in the saturated and unsaturated zones of subsoil. Some recent studies indicate that, under laboratory conditions, samples from aquifers and from the unsaturated zone (generally down to 1·5 m depth) often have a certain ability to degrade pesticides.[1–4] This degradation, however, occurs at lower rates than at the surface.

In surface soils and subsoils, the dissipation of atrazine can also be due to chemical processes. The chemical transformation of atrazine occurs by dechlorination of the triazine ring giving the hydroxylated derivative, hydroxyatrazine.[5–8] This reaction occurs mostly under acidic conditions and can be catalysed by soil organic matter.[5]

It is widely known, however, that the biotransformation of atrazine is the most effective process for its degradation in the soil environment. This process is initiated by *N*-dealkylation giving rise to deethylatrazine and, to a lesser extent, deisopropylatrazine.[9,10] Typically this first step in the biotransformation of atrazine follows first-order kinetics with a half-life of one to two months. Further transformation of the atrazine residues occurs more slowly, the triazine ring being resistant to degradation by soil micro-organisms.[11,12]

More recently it was shown that microbial isolates could mineralize atrazine at a high rate,[13–19] although, to date, this mineralization has been observed only when the micro-organisms were grown on specific culture media containing adapted organisms or in soils

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‡ To whom correspondence should be addressed.

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inoculated with these adapted organisms. It has been claimed that the first step of this rapid mineralization of atrazine is dechlorination to hydroxyatrazine.[13,15] Until recently, however, it was thought that bacteria were unable to produce the specific enzymes responsible for dechlorination of the triazine ring. Some strains of *Pseudomonas* sp. have been found to carry out this dechlorination but only with *N*-dealkylated triazines and not with the parent atrazine.[20] It was only in 1993 that Mandelbaum *et al.*[21] isolated soil bacteria that were able to transform atrazine to hydroxyatrazine rapidly, even after re-inoculation in a soil at neutral pH. Previous work by these authors[14] had shown that this dechlorination reaction was the first step in the processes leading to complete mineralization of atrazine. It seems that a specific DNA fragment encodes atrazine dechlorination and hybridization studies suggest that this gene is widespread in nature.[22]

The aim of the present study was to follow, under laboratory conditions, the degree of persistence of atrazine in samples taken from the soil and subsoil of various maize fields which had been treated every year with this herbicide for more than five years. It was expected, indeed, that a more rapid transformation and mineralization of atrazine could be found in natural soils that had been treated repeatedly with this herbicide than in those from fields which had not. To date, there is no report of accelerated degradation and/or mineralization of atrazine in 'natural' soils or subsoils.

2 MATERIALS AND METHODS

2.1 Selection of sites

For the selection of the sites to be sampled the criteria used were those of intensive maize cropping during several years with repeated atrazine applications during each of the previous years and vulnerable aquifer (shallow water table, sandy sub-soil, drinking water extraction).

The two sites selected were situated in Central Belgium in water catchment areas for the production of drinking water (aquifers in sandy material from the tertiary era). One site (Korbeek-Dijle, KBD) is in a rural area and the other (Louvain-la-Neuve, LLN) is in an urban area. On each site, the first boreholes (KBD1, KBD2 and LLN1) were drilled in December 1993 in fields which had been cropped for at least 10 years with maize and treated nearly every year with atrazine. Later, in June 1994, additional boreholes were drilled on both sites in maize fields (KBD3, KBD4, LLN2) as well as in a non-cropped area (LLN3), all of them situated less than one kilometre away from the first boreholes. The depth of the boreholes varied between 5 and 20 m and the water table was reached in four holes.

2.2 Sampling of soil

The samples were obtained using a dry percussion coring technique developed by the Institute of Hydrology (Wallingford, Oxford, UK) in conjunction with the British Geological Survey.[1] Steel tubes (0·5 m long × 100 mm diameter) were forced into the ground using a small site rig which applied blows from a weight suspended on a steel cable. When removed from the hole, the tubes were sealed at both ends, stored at 5(±2)°C and sent to the laboratory within 10 h after extraction from the hole. The incubation studies were carried out as soon as possible (not more than two days after sampling and storage under cool conditions).

Soil material was sampled from the cores in order to determine moisture content, pH of a slurry of one part soil in five parts water, organic matter (Walkey–Black), particle size distribution and the number of colony-forming micro-organism units (CFU) after incubation on Tryptic Soy Agar medium (3 g litre^{-1}).

2.3 Soil incubation studies

For the soil incubation studies, 20 replicate samples (10 g) of sediment were taken from each core after removal of the outer 2 cm to avoid contamination of the sampled material. Atrazine (4·5 mg kg^{-1}) and sterile water (final water content 45% of soil dry mass) were added and the vials were then incubated at 25°C in sterile containers. At the start of the experiment (i.e. 12 h after adding atrazine) and at various time intervals thereafter (see Figs 1 and 2), atrazine was extracted from some containers with acetonitrile (20 ml per 10 g of fresh soil) over a period of 12 h. After centrifugation and filtration, the atrazine content was determined by HPLC (C18 column from Alltima, methanol + water (75 + 25 by volume) as eluent, 1 ml min^{-1} flow rate and detection with a UV detector at 220 and 260 nm).

Parallel experiments were run with uniformly [*ring-U*-^{14}C]atrazine in which sediments (20 g) corresponding to specific depths were incubated with atrazine (150 μg kg^{-1}; specific activity 2·15 GBq mmol^{-1}) in sterile bottles equipped with a reservoir containing sodium hydroxide in order to trap the [^{14}C] carbon dioxide evolved. This solution was renewed after different time intervals. The radioactivity trapped was measured after mixing the sodium hydroxide sample with the scintillating cocktail Rialuma (Lumac) using the liquid scintillation counter Tricarb (Canberra Packard) with the usual correction for quenching.

It is recognised that 25°C is a relatively high temperature for incubation studies, especially with subsurface-samples, but it was set by the coordinator of the project as a standard for all laboratories taking part in the study (see Acknowledgements). It has been established (data not shown) that results can be extrapolated to other temperatures using standard procedures.

3 RESULTS AND DISCUSSION

3.1 Characterization of the soil samples

Table 1 presents the physicochemical properties of the samples taken from two characteristic boreholes, KBD1 and LLN2. The pH is mostly neutral to alkaline due to the presence of calcium carbonate incrustations in the sediments; only in the surface horizon of LLN2 are lower pH values found. This can be explained by the repeated growing of maize on this plot without chalk amendment. Silt predominates (62·5–85·9%) in the upper horizons of KBD1 but below 1·60 m the sand fraction becomes more important. In LLN2, there is relatively less silt in the upper horizons (14·9–67·2%) but it is only below 4·15 m that the sand fraction

TABLE 1
Major Characteristics of Soil Samples taken from the Two Boreholes

Depth (*cm*)		*pH*[a]	*Moisture content* (%)	*OM* (%)	*Particle size* (%)			*CFU* (g^{-1})	DT_{50} (*days*)
					Sand	*Silt*	*Clay*		
Louvain-la-Neuve 2 (LLN2)									
0–10		6·2	14·3	1·19	70·7	23·8	5·5	1·2E6	27
16–26		5·6	13·3	1·15	58·4	35·5	6·1	1·0E6	25
37–52		7·1	19·5	0·50	68·3	26·2	5·5	1·2E6	156
98–113		7·2	20·0	0·21	69·4	25·7	4·9	1·0E5	>450
157–172		7·2	23·5	0·21	62·6	31·6	5·8	7·0E4	>450
216–231		8·4	23·3	0·22	62·8	14·9	22·3	1·2E5	>450
279–294		8·4	23·5	0·22	8·5	67·2	24·3	1·0E4	—[c]
341–356	S[b]	8·2	17·6	0·17	56·9	21·0	22·1	7·0E4	—
400–415	S	8·4	16·8	0·11	63·3	7·2	29·5	5·0E4	—
455–470		8·5	4·9	0·08	89·4	1·3	9·3	2·0E3	—
509–524		8·0	4·1	0·11	91·7	0·9	7·4	0	—
570–587	I	8·0		0·34	73·6	12·2	14·2	1·0E3	22[d]
637–652		8·2	3·7	0·03	91·3	3·4	5·3	0	—
702–717		8·1	4·3	0·05	91·0	3·7	5·3	0	—
767–782		7·7	24·0	0·02	76·6	13·0	10·4	0	—
842–837		7·8	16·3	0·03	82·6	8·5	8·9	0	—
962–977		7·9	13·6	0·02	87·8	3·6	8·6	0	—
1092–1107		8·1	6·8	0·03	90·7	2·2	7·1	0	—
1231–1246	S/I	7·7	6·4	0·03	91·6	2·7	5·7	1·5E2	—
1357–1372		8·3	7·0	0·05	91·4	2·6	6·0	1·0E1	—
1492–1507	S	8·0	7·6	0·02	92·4	0·9	6·7	1·0E1	—
1622–1637		8·3	4·7	0·02	94·4	0·6	5·0	3·0E1	—
1686–1707	S	8·4	4·2	0·02	94·4	0·6	5·0	0	—
Korbeek-Dijle 1 (KBD1)									
0–10		8·7	22·5	1·02	11·2	85·9	2·9	3·4E6	5–10
10–20		8·6	22·5	1·02	9·2	84·9	5·9	2·5E6	30–60
38–53		8·6	20·2	0·21	23·2	72·1	4·7	3·6E4	>200
101–116		8·6	21·2	0·17	32·0	62·5	5·5	4·6E4	—
160–175		8·9	10·1	0·10	87·2	8·8	4.0	0	—
219–234	S	9·0	7·0	0·03	94·7	3·2	2·1	3·0E3	—
284–299		9·4	4·2	0·03	98·1	0·9	1.0	0	—
368–383		9·2	11·6	0·10	94·2	3·1	2·7	0	—
440–455		9·2	12·6	0·10	82·1	6·4	11·5	0	—
478–493		9·5	2·6	0·10	82·9	7·0	10·1	0	—
530–540	S	9·4	6·5	0·03	85·6	4·5	9·9	0	14[d] or —
575–590		9·4	3·0	0·07	87·8	3·7	8·5	0	—
625–640		9·2	6·4	0·07	83·0	3.4	13·6	0	—
668–683		9·0	19·3	0·03	87·0	2·6	10·4	4·0E3	—
700–712	S/aq	9·1	23·9	0·10	89·8	0·6	90·4	5·4E4	14[c]

[a] In a slurry in water.
[b] aq,: aquifer sediments, S; presence of stones; I: iron components.
[c] Less than 10% dissipation after 200 days.
[d] Degradation starts after a lag phase of 7 to 20 days.

exceeds 75%. The organic matter content is close to 1% in the surface horizons but drops with increasing depth. Slightly more organic matter (0·2%) seemed to be associated with samples with a higher silt fraction. As expected, the moisture content of the sediments sampled is greater when the silt fraction is important and it is also in these samples that the micro-organism content exceeds 10^3 CFU g^{-1}. In the deeper sediments the micro-organisms are generally undetectable except in some horizons where stones and/or iron components are present.

3.2 Persistence of atrazine in surface and subsurface soils

3.2.1 Surface soils

The DT_{50} values determined for atrazine in these samples were found to vary greatly from one soil to another (Fig. 1 and Table 2) but the most important observation is the very low values obtained with KBD1, KBD2 and KBD3. As far as we know, these DT_{50} values are much smaller than those normally found in an agricultural soil. Burkhard and Guth[23] found DT_{50} values for atrazine in two different soils incubated under laboratory conditions to be 53 days and 113 days, and the field DT_{50} under normal climatic conditions is given as 35–50 days.[24] One possible explanation for this short persistence could be microbial adaptation in these soils as a result of repeated use of atrazine. This seems to be particularly the case on the site of Korbeek-Dijle, at least for the soils characterized by high pH values (KBD1, KBD2 and KBD3).

TABLE 2
DT_{50} of Atrazine in Surface Soils from Various Maize Fields

Korbeek-Dijle (KBD)			*Louvain-la-Neuve (LLN)*		
Borehole	*pH*[a]	*DT_{50} (days)*	*Borehole*	*pH*[a]	*DT_{50} (days)*
KBD1	8·7	5–10	LLN1	5·5	37
KBD2	8·5	5–10	LLN2	6·2	27
KBD3	7·7	8	LLN3[b]	6·2	30
KBD4	4·8	33			

[a] In a slurry in water.
[b] Non-cultivated plot, never treated with pesticides.

3.2.2 Subsurface soils

In samples from depth <0·2 m, the disappearance of atrazine decreased progressively (Table 1). In general, there was little or no dissipation in samples from depths <1 m. For a few samples, however, there was a rapid decline in atrazine content after a lag phase of seven to 20 days (see LLN2: 470–587 cm and KBD1: 700–712 cm in Table 1 and Fig. 1). The high atrazine recovery (>90%) in the samples sterilized before incubation indicates that this decline is due to microbial activity. However, it is important to note that there is a large variability in the dissipation rates observed within the various subsamples of some specific cores (see for example KBD1: 530–540 cm). This occurs mostly when stones or iron components are present in the sample. In such samples, there was also a large variability in micro-organism counts: the closer to the surface of a stone, the larger the number of micro-organisms and the quicker the disappearance of atrazine (results not shown).

3.3 Mineralization of atrazine in the surface and subsurface soils

Additional experiments were carried out with [*ring-U-*^{14}C]atrazine in order to follow more closely the mineralization rate in the soil materials which were able to transform this herbicide rapidly. The results are presented in Fig. 2.

3.3.1 Surface soils

The production of [^{14}C]carbon dioxide occurred very rapidly in the case of KBD1 and this was accompanied by a very short persistence of atrazine (DT_{50} = 8 days). This indicates that the soil micro-organisms are able to cleave and mineralize the triazine ring. In contrast, min-

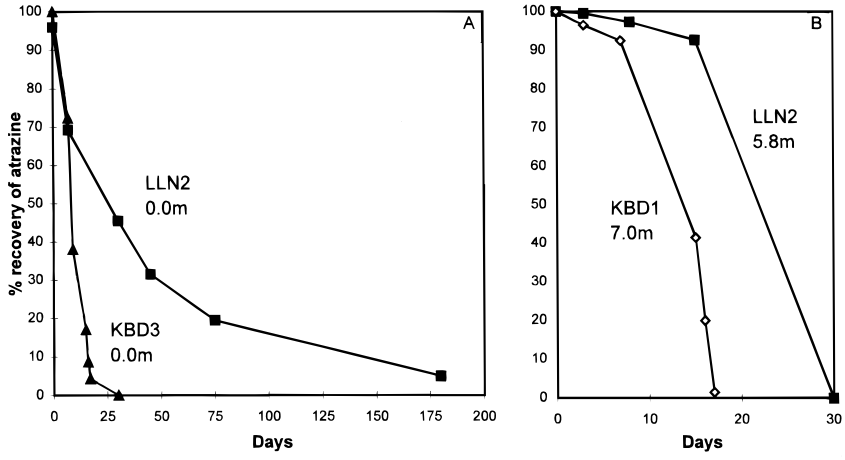

Fig. 1. Degradation of atrazine (4·5 μg kg^{-1}) (A) surface and (B) subsurface soil samples.

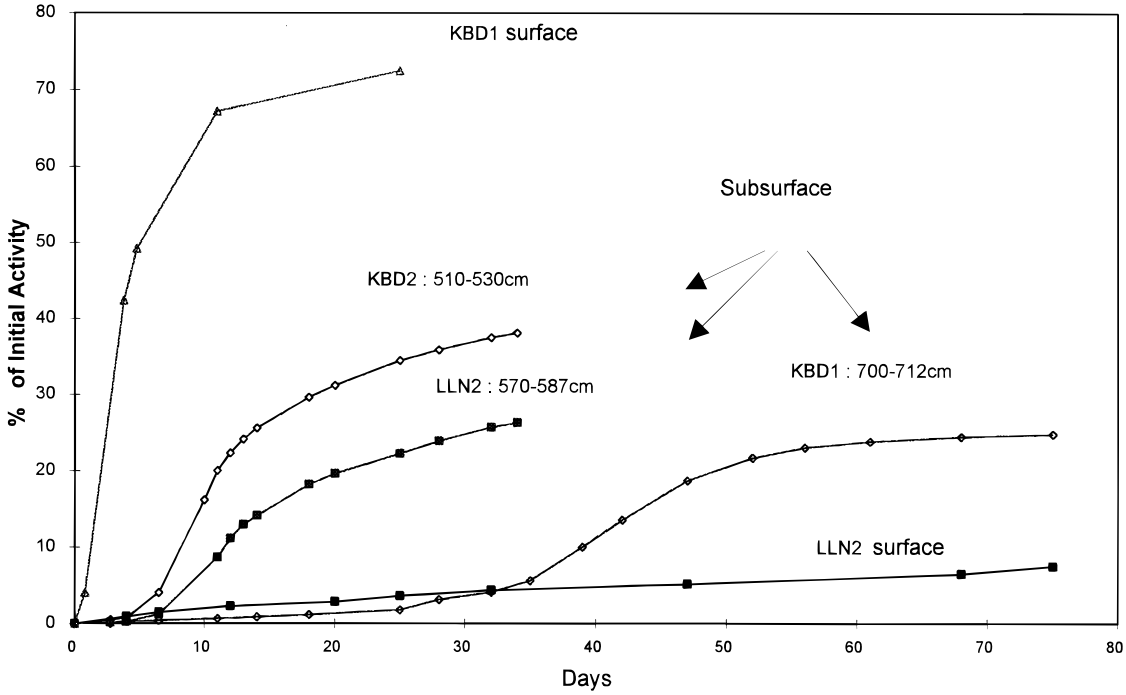

Fig. 2. Release of [^{14}C]carbon dioxide from [*ring-U*-^{14}C]atrazine (150 μg kg^{-1}) by surface and subsurface samples.

eralization into [^{14}C]carbon dioxide occurred much more slowly in the surface sample of LLN2 for which the DT_{50} value was 27 days.

3.3.2 Subsurface soils

Mineralization in LLN2 (depth: 570–587 cm) and in KBD2 (depth: 510–530 cm) was quite rapid. The latter material, which actually came from a borehole drilled 1 m away from KBD1, also contained stones and had a high micro-organism content (5×10^3 CFU g^{-1}); atrazine was degraded rapidly in this soil. The results obtained with KBD1 at 700–712 cm indicate that mineralization of atrazine seems also to be important in sediments of saturated zone, but only after a significant lag phase.

The pattern of mineralization differs between the rapidly degrading surface soil (KBD1) and the subsurface samples. First, a lag phase is observable in the subsoil, this being from about five days in the two samples taken in the unsaturated zone (KBD2: 510–530 cm and LLN2: 570–587 cm) to more than 25 days in the sample taken from the saturated zone (KBD1: 700–712 cm). This could be due to the fact that the number of degraders in subsoil might be low initially and that microbial growth is needed before degradation starts, as already pointed out by Moorman for other xenobiotic compounds.[25] Also, there is a plateau in [^{14}C]carbon dioxide production in samples from subsoil after 25–35% has been evolved, which is much less than in soil from the surface of KBD1 where more than 70% was produced. Thus, for the subsoil samples, one can assume either that more polar or bound residues are produced or that the radioactivity becomes associated to the microbial biomass in some manner.

4 CONCLUSIONS

From this study, carried out with samples taken from maize fields repeatedly treated with atrazine, it can be concluded that:

—a rapid degradation can take place in surface soils, in at least some of the collected soil materials which are characterized by a pH value $\geqq 7{\cdot}7$;
—this rapid degradation of the parent product seems to be linked with a high degree of mineralization of atrazine;
—biodegradation does not occur in the subsoil materials, except in some samples where stones or iron components shelter large numbers of micro-organisms;
—if degradation does occur in the subsoil samples, it starts after a lag phase of five to 25 days. The disappearance of atrazine is then rapid, with a high mineralization rate.

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